

Performance of the New VITEK 2 GP Card for Identification of Medically Relevant Gram-Positive Cocci in a Routine Clinical Laboratory

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The VITEK 2 gram-positive (GP) identification card (bioMérieux, Marcy l'Etoile, France) has been redesigned to achieve greater accuracy in the identification of gram-positive cocci. A total of 43 biochemical tests, including 17 enzymatic tests, are present in the card and interpreted in a kinetic mode, for up to 8 h. The VITEK 2 database, used in conjunction with the GP identification card, allows the identification of 115 different taxa. A total of 364 strains of GP cocci (217 *Streptococcaceae* strains and 147 *Micrococcaceae* strains) belonging to 31 taxa were tested with the new VITEK 2 GP identification card. Of the 364 strains, 105 were taken from routine primary plating media. A total of 344 strains (94.5%) were correctly identified to the species level and 17 strains (4.7%) were identified with low discrimination, requiring additional tests, whereas 1 strain (0.3%) was incorrectly identified and 2 strains (0.5%) remained unidentified. Within 7 h of the start of incubation, more than 90% of all strains were identified. Of the 105 primary cultures, 97% were correctly identified to the species level, 2% were identified with low discrimination, and 1% remained unidentified. Identification performance data were independent of each of the three plating media used. It is concluded that the new VITEK 2 GP identification card provides reliable results for the identification of GP cocci under routine laboratory conditions.

Highly automated identification systems are nowadays widely distributed in many medium-to-high-throughput clinical microbiology laboratories. These systems improve the quality of patient care and enable more-cost-effective management of the same by enabling clinical microbiologists to identify medically relevant bacteria more rapidly and accurately (1, 2). An important measure of the value of a highly standardized commercial identification system must be the capability of the manufacturer to maintain or even improve the performance of an identification system over time. The new VITEK 2 gram-positive (GP) identification card (bioMérieux, Marcy l'Etoile, France) for identification of GP cocci was created in recent years as research and development related to the VITEK 2 instrument continued. The rationale for designing the new VITEK 2 GP identification card was to broaden the VITEK 2 database while maintaining the quality of the identification results in the routine clinical laboratory. The GP identification card contains 43 tests (27 tests that had been included in the previous card and 16 new tests), compared to 47 in the established VITEK 2 GP identification card (ID-GPC), and 115, instead of 51, taxa are covered by the new database corresponding to the GP identification card. While the ID-GPC tests are based on fluorescence technology, the GP identification card tests are based on colorimetric detection. Both the ID-GPC and GP identification card tests are subjected to measurements every 15 min, and the total incubation time is up to

approximately 8 h with the GP identification card, as opposed to 2 h with the ID-GPC.

The aim of the present study was to evaluate the newly developed VITEK 2 GP identification card in a routine clinical laboratory by a weighted laboratory profile (9).

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MATERIALS AND METHODS

Laboratory, strains, culture conditions, and identification. The study was performed at Gärtner and Colleagues Laboratories, an accredited reference laboratory that serves over 100 hospitals of all levels and over 3,000 physicians in private practice. The strains included in the present study were collected within a 3-month period. The number of strains per species was limited to a maximum of 45. A total of 105 strains of GP cocci were taken from primary isolation plates set up on Columbia sheep blood agar (BD, Heidelberg, Germany) in our routine clinical laboratory for various types of patient specimens (blood culture, wound swab, respiratory, and urine specimens, etc.). The other 259 strains came from primary isolation plates that had been stored at 4 to 8°C for less than 1 week. These strains were subcultured on Columbia sheep blood agar from BD ($n = 70$), Columbia sheep blood agar from bioMérieux ($n = 78$), or Trypticase soy blood agar (bioMérieux) ($n = 111$) for 18 to 24 h at 37°C before they were subjected to VITEK 2 analysis. All strains included in the present study came from unrelated patients, and consecutive cultures from the same patient were also excluded. The 364 strains used in this study were identified by conventional methods (10) as well as by VITEK 1 analysis with the GP identification card designed for use with that system. For identification by conventional methods, the following characteristics were tested: colony pigmentation, hemolysis, adherence to agar, colony odor, catalase and oxidase reaction, clumping factor test (bioMérieux), reaction(s) to Lancefield group streptococcal antisera (Oxoid, Basingstoke, United Kingdom), reaction(s) to pneumococcal antisera (bioMérieux), as well as susceptibilities to optochin and bacitracin. Discrepancies between the identifications obtained by conventional methods and VITEK 1 analysis and

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TABLE 1. Performance of the new VITEK 2 GP identification card by species and by family

Species or family	No. of strains that were ^a :				
	Tested	Correctly identified	Identified with low discrimination	Misidentified	Not identified
Species					
<i>Enterococcus avium</i>	3	3	0	0	0
<i>Enterococcus casseliflavus</i>	1	1	0	0	0
<i>Enterococcus durans</i>	5	4	1	0	0
<i>Enterococcus faecalis</i>	27	27	0	0	0
<i>Enterococcus faecium</i>	28	27	1	0	0
<i>Micrococcus luteus</i>	4	4	0	0	0
<i>Rothia mucilaginosa</i>	3	3	0	0	0
<i>Staphylococcus aureus</i>	45	45	0	0	0
<i>Staphylococcus capitis</i>	8	8	0	0	0
<i>Staphylococcus epidermidis</i>	34	33	1	0	0
<i>Staphylococcus haemolyticus</i>	29	25	3	1	0
<i>Staphylococcus hominis</i>	13	13	0	0	0
<i>Staphylococcus lugdunensis</i>	5	5	0	0	0
<i>Staphylococcus sciuri</i>	1	1	0	0	0
<i>Staphylococcus schleiferi</i>	1	1	0	0	0
<i>Staphylococcus simulans</i>	1	1	0	0	0
<i>Staphylococcus wameryi</i>	1	1	0	0	0
<i>Staphylococcus xylosus</i>	2	1	1	0	0
<i>Streptococcus agalactiae</i>	35	32	3	0	0
<i>Streptococcus anginosus</i>	4	3	1	0	0
<i>Streptococcus constellatus</i>	1	1	0	0	0
<i>Streptococcus dysgalactiae</i> subsp. <i>equisimilis</i>	22	14	6	0	2
<i>Streptococcus equi</i> subsp. <i>zooepidemicus</i>	1	1	0	0	0
<i>Streptococcus gallolyticus</i>	4	4	0	0	0
<i>Streptococcus lutetiensis</i>	1	1	0	0	0
<i>Streptococcus oralis</i>	2	2	0	0	0
<i>Streptococcus parasanguinis</i>	1	1	0	0	0
<i>Streptococcus pasteurianus</i>	3	3	0	0	0
<i>Streptococcus pneumoniae</i>	39	39	0	0	0
<i>Streptococcus pyogenes</i>	38	38	0	0	0
<i>Streptococcus salivarius</i>	2	2	0	0	0
Total	364	344 (94.5)	17 (4.7)	1 (0.3)	2 (0.5)
Families					
<i>Micrococcaceae</i>	147	141 (95.9)	5 (3.4)	1 (0.7)	0 (0.0)
<i>Streptococcaceae</i>	217	203 (93.6)	12 (5.5)	0 (0.0)	2 (0.9)

^a Values in parentheses are percentages.

those obtained by VITEK 2 analysis were resolved by using ID 32 STAPH and rapid ID 32 STREP galleries (both from bioMérieux) as well as by sequencing of 16S rRNA genes (which was necessary for a total of nine strains) as previously outlined (5).

New GP identification card and VITEK 2 instrument. A bacterial suspension was adjusted to a McFarland standard of 0.5 in 2.5 ml of a 0.45% sodium chloride solution with a VITEK 2 instrument (DensiChek; bioMérieux). The time between preparation of the inoculum and the card filling was always less than 30 min. The format of the GP identification card is the same as that of the ID-GPC, i.e., a 64-well plastic card which contains now 43 instead of 47 tests (see above). The GP identification card includes test for the following reactions: phosphatidylinositol phospholipase C, arginine dihydrolase (two tests), β -galactosidase, α -glucosidase, alanine-phenylalanine-proline arylamidase, L-aspartic acid arylamidase, β -galactosidase, α -mannosidase, alkaline phosphatase, L-leucine arylamidase, proline arylamidase, β -glucuronidase (two tests), α -galactosidase, L-pyrogutamic acid arylamidase, alanine arylamidase, tyrosine arylamidase, and urease. The GP identification card also tests acid production from the following substrates: amygdalin, xylose, α -cyclodextrin, sorbitol, galactose, ribose, lactate, lactose, N-acetyl-glucosamine, maltose, mannitol, mannose, methyl- β -D-glucopyranoside, pullulan, raffinose, salicin, sucrose, and trehalose. Finally, growth in 6.5% NaCl as well as tests for resistance to polymyxin B, bacitracin, novobiocin, O129, and optochin are also included in the GP identification card.

The GP identification card is a fully closed system to which no reagents have to be added. The card was put on the cassette designed for use with the VITEK 2 system, placed in the instrument, automatically filled in a vacuum chamber,

sealed, incubated at 35.5°C, and automatically subjected to colorimetric measurement (with a new reading head) every 15 min for a maximum incubation period of 8 h. Data were analyzed using VITEK 2 database version 4.01, which allows organism identification in a kinetic mode beginning 180 min after the start of incubation. In the kinetic mode, all 43 tests are individually interpreted using a first-level algorithm based on the color change of the reaction. Every 15 min, a second-level algorithm analyzes the biopattern (i.e., interpreted and not interpreted tests) and verifies if it is sufficient to give the final identification call. The second algorithm also checks, using a specific calculation, that the later results of not-yet-interpreted tests will not change the identification call.

Quality control strains. During the evaluation period the following quality control strains were checked at regular intervals: *Enterococcus casseliflavus* (ATCC 700327), *Enterococcus faecalis* (ATCC 29212), *Enterococcus saccharolyticus* (ATCC 43076^T), *Kytococcus sedentarius* (ATCC 27575), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus saprophyticus* (ATCC BAA-750), and *Streptococcus equi* subsp. *zooepidemicus* (ATCC 43079^T).

Reporting of results. Not the identification scores (*t* index, probability, likelihood, and confidence values) provided by the software but rather the interpretations provided by the software were taken into account. The four different result categories were: (i) correct identification (unambiguous correct identification to the species level); (ii) low level of discrimination (either identification to the genus level or a low level of discrimination between two or more species, including the correct species); (iii) no identification; and (iv) misidentification (the species identified with the GP identification card was different from that identified by the reference method).

TABLE 2. Strains identified with low discrimination or misidentified

Strain group	Reference identification (no. of strains)	Identification provided by the new VITEK 2 GP identification card
Strains with low discrimination	<i>E. durans</i> (1)	<i>E. durans</i>
	<i>E. faecium</i> (1)	<i>E. hirae</i>
		<i>E. gallinarum</i>
		<i>E. faecium</i>
	<i>S. epidermidis</i> (1)	<i>S. epidermidis</i>
		<i>S. hominis</i>
	<i>S. haemolyticus</i> (3)	<i>S. haemolyticus</i>
		<i>S. wamari</i>
	<i>S. xylosus</i> (1)	<i>S. gallinarum</i>
Misidentified strain	<i>S. agalactiae</i> (3)	<i>S. xylosus</i>
		<i>S. agalactiae</i>
		<i>S. constellatus</i>
		<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>
	<i>S. anginosus</i> (1)	<i>S. anginosus</i>
		<i>S. gordonii</i>
	<i>S. dysgalactiae</i> subsp. <i>equisimilis</i> (6)	<i>S. dysgalactiae</i> subsp. <i>dysgalactiae</i>
		<i>S. dysgalactiae</i> subsp. <i>equisimilis</i>
	<i>S. haemolyticus</i> (1)	<i>S. wamari</i>

RESULTS

Overall, we did not encounter any major technical problem using the VITEK 2 instrument and the GP identification card during the 3-month evaluation period. Quality control strains were correctly identified to the species level in every instance, demonstrating the reliability and reproducibility of the technique. The hands-on time remained the same for the GP identification card as for the ID-GPC.

Table 1 lists the performance of the GP identification card for the 31 taxa (18 belonging to the family *Streptococcaceae* and 13 belonging to the family *Micrococcaceae*) tested. Of the total of 364 strains (217 belonging to the family *Streptococcaceae* and 147 belonging to the family *Micrococcaceae*), 344 strains (94.5%) were correctly identified to the species level, 17 strains (4.7%) were identified with low discrimination, 2 strains (0.5%) remained unidentified, and 1 strain (0.3%) was misidentified. Identification results were even better when simple additional tests (see below) were applied to resolve the results for strains with low discrimination. Identification results were slightly better for strains of the family *Micrococcaceae* (95.9% correctly identified, 3.4% identified with low discrimination, 0.7% misidentified, and 0.0% not identified) than for strains of the family *Streptococcaceae* (93.6% correctly identified, 5.5% identified with low discrimination, 0.0% misidentified, and 0.9% not identified).

The distribution of all taxa tested was not equal but was weighted according to the frequency with which the different species are seen in a routine clinical laboratory. The eight most frequently isolated gram-positive cocci, namely, *E. faecalis*, *Enterococcus faecium*, *S. aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* represented 75.3% of all strains included in the present study. Of these, 96.7% were correctly identified and 2.9% were identified with low discrimination (only one strain [0.4%] was misidentified).

Minor differences in identification results were observed with the different culture media. Of the 175 strains tested on Columbia sheep blood agar from BD, 96.6% were correctly

identified, 2.3% were identified with low discrimination, 0.0% were misidentified, and 1.1% were not identified. Of the strains tested on Columbia sheep blood agar from bioMérieux, 88.5% were correctly identified, 10.3% were identified with low discrimination, 1.3% were misidentified, and 0.0% were not identified; of the strains tested on Trypticase soy blood agar, 95.5% were correctly identified, 4.5% were identified with low discrimination, 0.0% were misidentified, and 0.0% were not identified.

Table 2 lists the strains identified with low discrimination as well as the misidentified strains. The differentiation between *Enterococcus durans* (saccharose positive) and *Enterococcus hirae* (saccharose negative) is readily achieved. *Enterococcus gallinarum* is motile, but *E. faecium* is not. *S. epidermidis* ferments D-mannose, whereas *Staphylococcus hominis* is unable to do so. *S. haemolyticus* is urease negative but expresses pyrrolidonyl arylamidase activity, whereas *Staphylococcus warneri* is urease positive and pyrrolidonyl arylamidase negative. *Staphylococcus xylosus* is differentiated from *Staphylococcus gallinarum* by a negative reaction for D-raffinose fermentation. The β-hemolysis reaction of *Streptococcus agalactiae* is more discrete than that of *Streptococcus dysgalactiae* subsp. *equisimilis*. In addition, *S. agalactiae* is positive in the Voges-Proskauer reaction, but *S. dysgalactiae* subsp. *equisimilis* is not. *Streptococcus constellatus* does not hydrolyze hippurate and exhibits the typical diacetyl odor, whereas *S. agalactiae* is hippurate positive and does not produce the typical “*Streptococcus milleri*” odor. *Streptococcus anginosus* is Voges-Proskauer positive, but *Streptococcus gordonii* is not. *S. dysgalactiae* subsp. *equisimilis* is beta-hemolytic, whereas *S. dysgalactiae* subsp. *dysgalactiae* is not.

Table 3 gives a detailed report on the exact time required for final identification of the strains tested. Over 90% of all strains were identified within 7 h. No significant difference was observed in the times to final identification of *Micrococcaceae* and *Streptococcaceae*.

Table 4 lists the identification results when gram-positive cocci from primary plating media were tested. As with the

TABLE 3. Time to final identification by VITEK 2 analysis with the new GP identification card

Species or family	No. of strains tested	% of identifications completed in under:						
		2 h	3 h	4 h	5 h	6 h	7 h	8 h
Species								
<i>Enterococcus avium</i>	3					100	100	100
<i>Enterococcus casseliflavus</i>	1					100	100	100
<i>Enterococcus durans</i>	5					60	60	100
<i>Enterococcus faecalis</i>	27		70	100	100	100	100	100
<i>Enterococcus faecium</i>	28				75	96	96	100
<i>Micrococcus luteus</i>	4				50	100	100	100
<i>Rothia mucilaginosa</i>	3				67	100	100	100
<i>Staphylococcus aureus</i>	45			71	91	96	100	100
<i>Staphylococcus capitis</i>	8					75	100	100
<i>Staphylococcus epidermidis</i>	34				32	82	91	100
<i>Staphylococcus haemolyticus</i>	29				52	76	83	100
<i>Staphylococcus hominis</i>	13				54	77	92	100
<i>Staphylococcus lugdunensis</i>	5				40	100	100	100
<i>Staphylococcus sciuri</i>	1					100	100	100
<i>Staphylococcus schleiferi</i>	1					100	100	100
<i>Staphylococcus simulans</i>	1						100	100
<i>Staphylococcus wamari</i>	1					100	100	100
<i>Staphylococcus xylosus</i>	2					50	50	100
<i>Streptococcus agalactiae</i>	35				14	86	89	100
<i>Streptococcus anginosus</i>	4				25	75	75	100
<i>Streptococcus constellatus</i>	1							100
<i>Streptococcus dysgalactiae</i> subsp. <i>equisimilis</i>	22					41	45	91
<i>Streptococcus equi</i> subsp. <i>zooepidemicus</i>	1					100	100	100
<i>Streptococcus gallolyticus</i>	4						100	100
<i>Streptococcus lutetiensis</i>	1					100	100	100
<i>Streptococcus oralis</i>	2				50	100	100	100
<i>Streptococcus parasanguinis</i>	1				100	100	100	100
<i>Streptococcus pasteurianus</i>	3					100	100	100
<i>Streptococcus pneumoniae</i>	39		3	10	90	95	97	100
<i>Streptococcus pyogenes</i>	38			37	76	92	95	100
<i>Streptococcus salivarius</i>	2		50	50	100	100	100	100
Total	364	0.0	5.8	21.1	55.3	85.2	90.7	99.4
Families								
<i>Micrococcaceae</i>	147	0.0	0.0	21.8	54.4	85.0	93.2	100
<i>Streptococcaceae</i>	217	0.0	9.7	21.2	56.2	85.3	88.9	99.1

overall study, a weighted distribution of isolates was tested. The results for testing of cocci from primary isolation plates were slightly better (97.1% correctly identified) than the results of the overall study.

DISCUSSION

To the best of our knowledge, this is the first study on the performance of the GP identification card in a routine clinical laboratory. Overall, we were impressed by the performance of the system, since more than 94% of the isolates were correctly identified to the species level without application of any further additional tests. This performance is clearly above the demanded 90% accuracy level that has been discussed by some authorities in the field of commercial clinical microbiology device evaluations. The taxonomy used in the database was up-to-date (e.g., *Streptococcus gallolyticus* instead of *Streptococcus bovis* biotype I [12] and *Streptococcus pasteurianus* instead of *S. bovis* biotype II.2 [11]), which is not always the case

for other commercial identification systems designed for GP cocci. In addition, we are not aware of any other commercial identification system for GP cocci claiming to cover so many different taxa.

Considering the broad distribution and use of the VITEK 2 instrument in routine clinical microbiology laboratories worldwide, surprisingly few evaluations of the ID-GPC have been published (Table 5). The present study is the third largest on VITEK 2 test cards for identification of GP cocci, and it is the largest with regard to the number of individual taxa that have been included. Despite the extended incubation and reading time, as well as the larger database, the results of our present evaluation were comparable to the results of the two other major studies on VITEK 2 test cards for identification of GP cocci (8, 13). It is important to note that the extension of the database did not lead to poorer identification results. The identification of *E. faecium* (96.4% of the strains correctly identified in the present study in contrast to only 71.4% in a previous study [8]) as well as the identification of *S. hominis* (100% of the strains correctly identified in the present study in contrast to only 65.6% in a previous study [13]) have been significantly improved. If the kinetic mode is used, the majority of the results obtained with the GP identification card become available about 3 to 4 h later (Table 3) than with the ID-GPC. However, this may be of no consequence for microbiology laboratories not operating on a 24-h schedule. In summary, our data indicate that the GP identification card fulfills the needs of a routine clinical microbiology laboratory serving outpatients as well as hospitalized patients. Regarding the cost effectiveness we cannot at present comment on the GP identification card because the pricing of the GP was not available to us at the time of writing this article.

Finally, it is recommended that other evaluations may include a larger number of strains from primary isolation plates in order to study whether the VITEK 2 system in conjunction with the GP identification card performs as acceptably as in the present study when a limited number of first-isolation strains is used. A pure stress test evaluation that includes nearly all taxa present in the database, in particular, the recently delineated,

TABLE 4. Performance of the new VITEK 2 GP identification card applied to cocci from primary isolation plates

Species	No. of strains that were ^a :				
	Tested	Correctly identified	Identified with low discrimination	Mis-identified	Not identified
<i>Enterococcus avium</i>	1	1	0	0	0
<i>Enterococcus faecalis</i>	26	26	0	0	0
<i>Enterococcus faecium</i>	3	3	0	0	0
<i>Rothia mucilaginosa</i>	1	1	0	0	0
<i>Staphylococcus aureus</i>	26	26	0	0	0
<i>Staphylococcus epidermidis</i>	7	7	0	0	0
<i>Staphylococcus haemolyticus</i>	2	1	1	0	0
<i>Streptococcus agalactiae</i>	9	9	0	0	0
<i>Streptococcus dysgalactiae</i> subsp. <i>equisimilis</i>	3	1	1	0	1
<i>Streptococcus pneumoniae</i>	11	11	0	0	0
<i>Streptococcus pyogenes</i>	15	15	0	0	0
<i>Streptococcus salivarius</i>	1	1	0	0	0
Total	105	102 (97.1)	2 (1.9)	0 (0.0)	1 (1.0)

^a Values in parentheses are percentages.

TABLE 5. Evaluations of identification cards for gram-positive cocci on the VITEK 2 instrument

Reference	Yr of publication	Card evaluated	No. of strains	% of strains that were:			
				Correctly identified	Identified with low discrimination	Misidentified	Not identified
6	2000	ID-GPC	150	87.3		12.7	
8	2002	ID-GPC	384	91.4	4.4	1.6	2.6
7	2002	ID-GPC	99	71.7		20.2	8.1
13	2003	ID-GPC	405	95.6	3.2	0.2	1.0
Present study	2005	GP identification card	364	94.5	4.7	0.3	0.5

infrequently encountered catalase-negative, GP cocci (3, 4) is also recommended, since our evaluation covered only a portion of the taxa in the database, though it of course comprised the most frequently found and clinically relevant GP cocci. As with the ID-GPC, it is encouraged and expected that studies by other authors will evaluate the performance of the GP identification card in different countries and under different laboratory conditions.

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